Indian J Physiol Pharmacol 2016; 60(4) : 386–391

Original Article

# Effects of *Morus Nigra* Leaves Extract on Insulin Secretion from Isolated Islets of Langerhans in Male Mouse

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## Abstract

**Objective:** Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose. The medicinal plants are important due to lower side effects and cost than drugs. Herbal remedies have long been used in the treatment and prevention of diabetes mellitus. The purpose of the present study was to evaluate the effects of *Morus nigra* leaves extract (MNE) on insulin secretion from beta-cells.

**Methods:** *Morus nigra* leaves were collected, dried, and powdered. The dry powder was extracted with 80% methanol. Extracts with different concentration (0.1%, 0.5%, and 1%) and glibenclamide (1 and 10μM) were applied on islets of Langerhans. The pancreatic islets from normal mice were isolated by collagenase digestion method. Insulin secretion from handpicked islets was evaluated in the static incubation system.

**Results**: Insulin release was significantly increased at 16.7 mM in comparison with 2.8 mM glucose concentration ($P<0.05$). A significant increase in insulin secretion was observed with MNE (1%) at glucose 2.8 mM ($P<0.001$). Glibenclamide significantly increased insulin secretion at glucose 2.8 mM and 16.7 mM ($P<0.01$).

**Conclusion:** Our findings demonstrated that MNE increased insulin secretion on beta cells in a dose-dependent manner. This implies that the consumption of MNE can be helpful in reducing the insulin resistance in diabetes.

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(Received on April 12, 2016)

## Introduction

Type II diabetes is a multi-factorial disease that is characterized by a reduction in the ability of the pancreas to make enough insulin to keep normal glucose homeostasis, because of impairment of function in pancreatic B-cells (1). It is frequently associated with a cluster of pathologies including

obesity, hypertriglyceridemia, impaired glucose tolerance, dyslipidemia, cardiovascular diseases, raised indicators of oxidative stress, and insulin resistance (2-5). Langerhans pancreatic islets release important hormones that are necessary for controlling blood glucose. In recent years, the study on Langerhans pancreatic islets is one of the main goals of the researchers in the field of diabetes treatment (6). The management and control of diabetes by chemical drugs without any side effects is still a challenge. Although there are a variety of commercially available drugs for the cure of diabetes, such as glibenclamide, these chemical drugs cannot be used long-term because of their unwanted side effects (7). Therefore, a lot of effort has been undertaken to find new antidiabetic agents from various sources, particularly medicinal plants because they are effective with fewer side effects and have relatively low cost. Approximately 800 plants worldwide have been found to support antihyperglycemic effects (8). Medicinal plants have been used worldwide for years to treat symptoms of diabetes. Also, animal studies have reported that some of these medicinal plants are even more effective than the chemical drugs currently used in diabetic animals (9).

Antioxidant, antidiabetic and androgenic properties of *Morus alba* leaf extract in diabetic rats have been shown (10). The *Morus nigra* belongs to the genus Morus of the family Moraceae. *Morus nigra* leaves are well-known as a traditional medicine with antihyperglycemic action (11). *Morus nigra* fruit provides a protective action against peroxidative damage to biomembranes and biomolecules (12). *Morus nigra* is rich in polyphenols, alkaloids, anthocyanins, and flavonoids that have been suggested to be responsible for its health benefits (13-15).

However, at least to our knowledge, there have not been previous reports on the effect of MNE on insulin secretion from beta-cells. The aim of the present study was to examine the effect of MNE administration (with different concentrations) on insulin secretion from isolated islets and also we compared the results of glibenclamide effect with MNE on insulin secretion from beta-cell mass.

## Materials and methods

### Animals

Male Nmari mice weighing 20-25 g, treated in accordance with the principles and guidelines for animal care of Ahvaz Jundishapur University of Medical Sciences (AJUMS), were used throughout this study. The animals were housed at 20-24ºC under a 12 h light-12 h dark cycle. They had free access to food and water.

### Experimental procedure

Islets were isolated by a modification of the protocol described originally by Lacy in 1967, using enzymatic digestion with a relatively crude preparation of collagenase. The first step of the protocol was to remove the pancreas from the animal and cut it off from exocrine pancreatic tissues (16). We used two animals (20-25 g) for islet isolation each day. Numbers of islets of Langerhans obtained depended on the weight and age of the animals. High yields of the islets could be taken from young animals. To remove the pancreas from the animal we made a V-incision in the genital area, then the bowel was moved to the left side of the dissected animal. Pancreas could be distinguished from the surrounding fat, which is yellow-brownish in color. Pancreas is located close to the spleen, which is a good marker to find the pancreas. Forceps were used to keep the spleen and the pancreas was cut away from the surrounding tissue, where it was attached at the small intestine. The pancreas was removed from the body and placed into 100 mL of buffered saline solution in a petri dish and other non-pancreatic tissues, such as retained fat and lymph nodes, were cut away. The size of the pancreas increased by injecting 10 mL buffered saline solution in the all folds of the pancreas so a larger surface area was created. The pancreas was placed into a 50 mL glass beaker and chopped using scissors until all the pieces were approximately the same size (1 mm × 1 mm). The contents were transferred to the 15 mL falcon tube, then centrifuged for 5 min at 100 g. Supernatant, which contains fat, was removed and the pellet was transferred to 15 mL conical tubes. Collagenase type IV was used for

digesting the pancreas (1-2 mg/pancreas) in a 1:1 mixture of buffered saline solution in order to release islets from exocrine pancreas tissue. Then, conical tubes were placed in a shaking water bath at 37°C and it was shaken at 800 oscillations per minute; normally shaking for 8-10 min is sufficient to release free islets from the exocrine tissue of the pancreas. Using 20 mL of cold buffer, digestion is stopped. To remove the collagenase in the solution, it was centrifuged at 500 g for 5 min and the supernatant was aspirated. Then, the pellet was resuspended into the 15 mL buffer isolation. The resuspended pellet was removed and transferred to a 90 ml black petri dish. The black background of the petri dish made a tremendous contrast to see the islets under a dissection stereomicroscope. Islet purification was performed by handpicking with a sampler. The process of handpicking islets is a labor intensive and time-consuming method, but it gives islet preparations with the highest purity.

#### Buffered saline solution

Hank's buffer solution was used in this study, which was made from several solutions. We have explained this in detail below:

#### Stock solution A (in mM):

115 NaCl, 5 KCl, 10 $NaHco_{3,}$ 1.1 $MgCl_2$, 1.2 $NaH_2po_{4,}$ 25 HEPES

#### Stock solution B (in mM):

2.56 $CaCl_2$

This solution can be kept for one month at room temperature. We prepared and stored a stock solution at 4°C. To prepare Hank's solution, 10 ml solution A, 10 ml solution B and 80 ml distilled water were mixed for a 100 ml working solution. Then, albumin 0.5% and 2.8 or 16.7 mM D-glucose were added to make low and high concentrations (pH=7.4).

#### Preparation of hydroalcoholic *Morus nigra* leaves extract

*Morus nigra* leaves were handpicked from trees at Ahvaz Greengrocery in spring. Then they were powdered by the grinder. Fifty grams of powder of *Morus nigra* leaves were mixed with 80% methanol for 72 h, using a macerated method. The mixture was filtered with Whatman No. 1 filter paper. The solvent of the filtrate was evaporated at ambient temperature and the extracted powder was kept at 4°C in the lab refrigerator until use (17).

#### Study design

We prepared 12 test groups (each group contained eight isolated islets). Test groups were incubated with a buffered saline solution containing glucose 2.8 and 16.7 mM with or without drug or extract. Group1: glucose 2.8 mM (without drug or extract); Group 2, 3: glucose 2.8 mM added by glibenclamide 1 and 10μM; and Group 4, 5, 6: hydroalcoholic MNE 0.1%, 0.5% and 1%. Group7: glucose 16.7 mM (without any drug or extract); Group 8, 9: glucose 16.7 mM added by glibenclamide 1 and 10 μM; and Group 10, 11, 12: hydroalcoholic MNE 0.1%, 0.5%, and 1%.

#### Biochemical analysis

Islets were incubated in a 95%$o_2$-5%$co_2$ incubator within 30 min of isolation, then the supernatant was separated carefully by pipette and insulin secretion was measured by ELISA kit. Of supernatant, 50 μL was used for the measurement of insulin by ELISA (DiaMetra-ELISA-Kit). In this assay, the cross-reactivity with proinsulin and C-peptide was not detectable and the minimal detectable concentration (M.D.C.) of insulin was 1 μIU/ml. The intra- and inter assay variations were 3% and 12%, respectively.

#### Statistical analyses

The data were expressed as the mean±standard error of the mean (S.E.M). One-way analysis of variance was performed and supported by an less significant difference (LSD) test. A statistical P-value less than 0.05 was considered significant.

## Result

In this study, insulin secretion was significantly

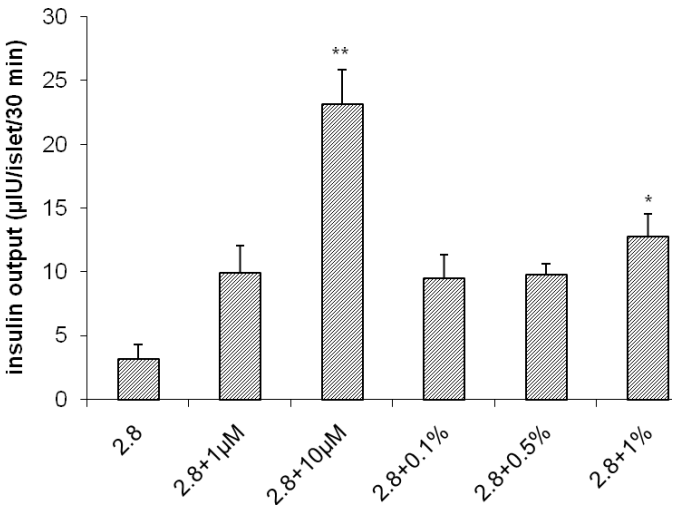


Fig. 1 : Effects of different concentrations of hydroalcoholic Morus nigra leaves extract and glibenclamide on insulin secretion from Beta cells at 2.8 mM glucose. (*p<0.05, **p<0.01)
*= Significant difference between glucose 2.8mM group with other groups.

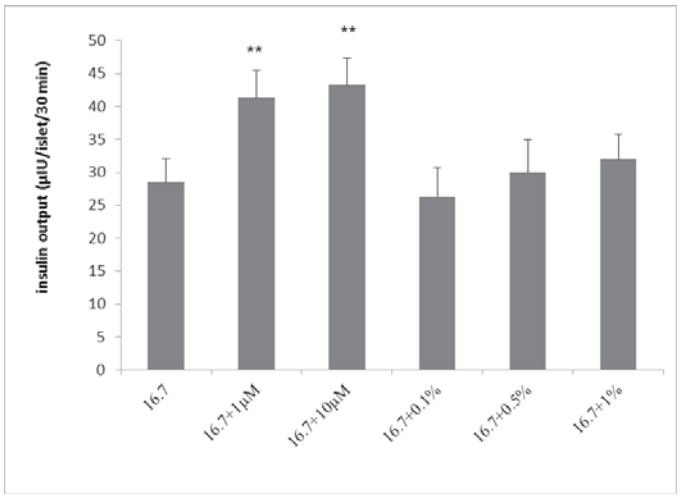


Fig. 2 : Effects of different concentrations of hydroalcoholic Morus nigra leaves extract and glibenclamide on insulin secretion from Beta cells at glucose 16.7 mM. (**p<0.01)
*= Significant difference between glucose 16.7 mM group with other groups.

increased at 16.7 mM (28.49±3.5 µIU/islet/30 min) in comparison with 2.8 mM (3.17±1.1µIU/islet/30 min) glucose concentration (p<0.001) (Fig. 1 and Fig. 2).

Our findings presented that glibenclamide significantly increases basal insulin secretion in pancreatic islets at glucose 2.8 mM and 16.7 mM, as compared with control islets. Glibenclamide had less activity at glucose 16.7 mM in comparison with 2.8 mM glucose (Fig. 1 and Fig. 2 P<0.01).

Incubation of pancreatic islets with different concentrations of *Morus nigra* extract and glucose 2.8 mM increased insulin secretion. A significant increase in insulin secretion was observed at high concentration (1%) of extract (Fig. 1, P<0.05).

Significant effects of different concentrations of the extract on insulin secretion were not found in concentrations of glucose 16.7 mM. Glibenclamide was more effective than *Morus nigra* extract leaves at high glucose concentrations (Fig. 2).

**Conclusion**

Our studies demonstrated that insulin secretion at 16.7 mM glucose concentrations increased significantly, compared with that at 2.8 mM glucose concentrations. In the present study, MNE acted on islet-isolated and significantly increased insulin secretion when glucose concentration was low. This is in agreement with the study of Ahmed et al. that showed that antidiabetic activity of the *Morus nigra* leaf extracts in streptozotocin-diabetic rats and administration of a leaf extract decreased glucose increased insulin levels (18). Also, Wang et al. demonstrated that *Morus nigra* L is used in traditional medicine to treat diabetes. Two new flavonoids were obtained as well as from its leaves (19). Iqbal et al. presented that high levels of phenolic compounds, antioxidant activity and radical scavenging MNE have the effect of potentially preventing disease (20).

MNE had no effect on insulin secretion of islets in a high glucose concentration. It has no effect on insulin secretion in the presence of 16.7 mM glucose concentration, which might relate to glucose toxicity and the presence of high glucose concentrations as a potent activator of insulin secretion interfering with MNE, which is consistent with our previous study (21).

Previous studies reported that changes of glucosidase inhibitory activity in leaves were

occurring among mulberry polyploidy (22). A study by Khazaei et al. showed that the symptoms of diabetes and neuropathy were improved by MNE. It is possible that these extracts have similar behavior in reducing diabetes symptoms and eliminating neuropathy like anesthetic and analgesic drugs. (23). It has been cited that the extract derived from *Morus nigra* leaves has antidiabetic effects (24). It seems that special attention to these natural compounds is essential to search for new therapeutic agents. (25). Therefore, the hypoglycemic effect of *Morus nigra* in the present study is in agreement with the results of previous studies that have showed the similar effect of this plant. One of the reports that was done on humans showed that adding the extract to the glibenclamide group did not increase the antidiabetic effect of this drug but neuropathy and symptoms of diabetes were improved by the extracts (26).

Glibenclamide increased insulin secretion in glucose concentrations of 2.8 and 16.7 mM, however, data showed that the increasing effect of glibenclamide on insulin secretion from beta-cells is more effective at low glucose concentrations than at high concentrations; this result is in agreement with other reports (27-29). Regarding the evidence that glibenclamide was less effective at high glucose concentrations, this is because the hypoglycemic effect of glibenclamide is covered by a high glucose concentration. From the results of this study it can be concluded that incubation of pancreatic islets with different concentrations of *Morus nigra* extract showed that insulin secretion increased significantly when at low glucose concentrations and high concentrations of extract, so from our result it can be concluded that extract of *Morus nigra* leaves has a hypoglycemic effect like sulfonylurea's agents on pancreatic beta cells because both of them could increase insulin secretion at low glucose concentrations. The insulin secretion doesn't change at low concentrations of extract. The results also showed that MNE couldn't increase insulin secretion when glucose concentration is high, which may be because at high glucose concentrations beta-cells are damaged, or it may because high glucose concentrations inhibited the insulin secretion effect of the extract. This implies that the consumption of MNE can potentially be useful in reducing insulin resistance in diabetes. Identification of antihyperglycemic compounds from *Morus nigra* and further studies for novel antidiabetic drug development are recommended. It can be concluded from this study that developed diabetes before the disease using beneficial plants to prevent the occurrence of this disease. Otherwise, in advanced diabetes, medicinal herbs such as *Morus nigra* can also be useful.

## Acknowledgments

The results presented in this work have been obtained from a student's thesis. This study was supported by the Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences (No. PRC-62).

**Conflict of interest**

The authors declare that there are no conflicts of interest.

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